2 MECHANICAL STIMULATION CONTROLS CANOPY ARCHITECTURE AND IMPROVES VOLUME

3 UTILIZATION EFFICIENCY IN BIOREGENERATIVE LIFE-SUPPORT CANDIDATE CROPS

4

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12 ABSTRACT

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16 *Key Words*

17 Thigmomorphogenesis, Plant Dwarfing, *Capsicum annum* 'California Wonder', Pepper,

18 Bioregenerative Life-Support

19

20 **ABBREVIATIONS**

21 VUE – Volume Utilization Efficiency

22 ALS – Advanced Life Support

23 MS – Mechanical Stimulation

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1. INTRODUCTION

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31 It has long been recognized that mechanical stimulation (MS; stress), such as wind action, rubbing,

32 constriction, shaking, and encounters with physical barriers can have a dramatic influence on

33 plant morphological development[1-5]. Jaffe (1973) demonstrated that daily MS to partially

34 mature internode tissue, applied by rubbing stem tissue between two fingers, could induce

35 dramatic reductions in internode length resulting dwarf phenotypes in a range of crop species.

36 Jaffe (1973) coined the term thigmomorphogenesis, (*thigma* being the Greek word for *touch*) to

37 describe these long term morphological responses to touch. Over the ensuing 40 years many

38 others have followed up on Jaffe's work, most notably Cary Mitchell's group at Purdue (West

39 Lafayette, IN, USA) and Janet Braam at Rice University (Houston, TX, USA). It is now known that

40 thigmomorphogenesis includes a wide range of responses including, but are not limited to,

41 shortening of internodes, stem thickening, reduced leaf expansion, changes in chlorophyll

42 content, and alterations in plant hormone levels[5-11].

43

44 A significant amount of thigmomorphogenesis research conducted during the 1980s-90s was

45 sponsored by the National Aeronautics and Space Administration (NASA)[1,4,10,12,13]. Given the

46 physical rigours of spaceflight it is important to understand how mechanical and vibrational

47 stimuli affected plant growth, and importantly, how MS could be used to counter the absence of

48 a gravity vector that may otherwise result in plants with leggy growth or being susceptible to

49 breakage[14]. The research findings were fairly consistent, at least in terms of the effects of MS on

50 plant architecture; MS results in shorter more compact plants[3,11,13,15]. These findings were

51 significant in that mass and volume are major limiting factors in the design and development of

52 bioregenerative life-support systems. Taking advantage of thigmomorphogenesis to produce

53 dwarf plant architectures, thereby reducing mass and volume requirements, is of interest.

54

55 Crops selected for use in bioregenerative systems need to conform to the many constraints of

56 spaceflight. As noted, a major constraint are the extremely limited real estate available for plant

57 production. Unlike most terrestrial agricultural applications that strive to optimize the use of a

58 given *area* of arable land, spaceflight agriculture requires researchers to maximize *volume* use

59   efficiency (VUE). This can be achieved through genetic manipulations, chemical interventions

60   (e.g., exogenous growth regulators), the selection of dwarf cultivars, and through specific

61   horticultural management practices utilizing standard crop species[16-20].   These dwarfing

62   mechanisms are now being combined with advances in light emitting diode (LED) systems which,

63   due to their cool operating temperature, allows for close proximity of the crop and light source,

64   enabling significant improvements in VUE.   Volume use efficiencies have improved to the point

65   that viable stacked or vertical agricultural production industries have emerged, in addition to

66   other applications such as molecular farming that often employ multi-layered or vertical

67   production architectures (Goto, 2012).

68

69   Public and private efforts are rapidly advancing both the notion and the technology required to

70   send humans to the Moon and Mars for extended periods. Bio-regenerative or advanced life-

71   support (ALS) systems utilizing plants and other biological machinery to sustain human life have

72   long been considered critical for such extended missions beyond low Earth orbit[21]. The plants and

73   associated microbial communities in these bio-regenerative systems provide, in whole or in part,

74   critical life-support services including food production, air revitalization (oxygen production and

75   carbon dioxide removal), and wastewater recycling[22,23]. Modifying the architecture of any given

76   crop, through such responses as thigmomorphogenesis, could help reduce the equivalent system

77   mass (ESM) of bio-regenerative systems ultimately leading to viable 'agriculture in space'

78   (Drysdale et al, 2003).

79

80   The objective of the presented study was to re-examine thigmomorphogenesis as a tool for

81   improving VUE in bioregenerative life-support and vertical agriculture system designs, while

82   adding to the knowledge base in this domain. The data obtained from the fruiting study were also

83   used to generate a rudimentary VUE model for vertical agriculture applications, given recent

84   advances (lighting) in controlled environment system technology.

85

86   **2. MATERIALS & METHODS**

87   Two experiments were conducted to determine the vegetative response, and fruiting response

88   of *Capsicum annum* 'California Wonder' to mechanical stimulation (MS). Response data were

89   used as a baseline for volume utilization efficiency calculations for hypothetical spaceflight and

90   vertical agriculture applications.

91

92   **2.1 Vegetative Response Study**

93   ***2.1.1 Plant Material Preparation and Growth Conditions****: Four seeds of *Capsicum annum*

94   'California Wonder' (Lake Valley Seed Company Inc., Boulder, CO) were sown in each of 18, 1.67L

95   pots containing a standard potting media (Fafard ProMix 2B, Sun Gro Horticulture Distribution

96   Inc., Agawam, MA), in which 8.3 g/L of 18-6-8 slow release fertilizer (Nutricote Total, Florikan

97   E.S.A. Corp., Sarasoda, FL) was incorporated. Plants were hand watered daily with deionized

98   water and supplemented with a half strength Hoagland's solution twice per week for the duration

99   of the trial. The chamber was maintained at a 23/20°C day/night temperature profile, a 16-h

100 photoperiod, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, constant relative humidity of 65%, and 800 ppm CO_2.

101

102 ***2.1.2 Layout***: Pots were randomly assigned to positions in a 3 x 6 grid within the growth chamber.

103 Treatment levels (1-control; 2-mechanically stimulated) were randomly assigned to each grid

104 position in a completely randomized design structure. The experiment was replicated to validate

105 the results.

106

107 ***2.1.3 Treatment Application***: Mechanical stimulation (MS) was initiated after a 1-week

108 acclimation period following transplanting into pots. Tightly wrapped cotton-tipped inoculation

109 sticks (InoculatorZ™, Biolog Inc., Hayward, CA) were used to apply gentle but firm strokes along

110 each side of the most recently developed internode at an application rate of 10 stroke per side

111 (total of 40). The internode was supported during the treatment by the placing two fingers on the

112 internode opposite to the point of MS. The MS was applied twice daily on weekdays and once

113 daily on weekends. Treatments were applied in the morning between 09:00-10:00 (2-3 h after

114 lights came on) and in the evening (16:00-17:00), for the duration of the experiment.   In order

115 to avoid confounding the MS dwarfing effect with the amount of incident light between the

treatments, the MS plants were placed on vertical risers, as needed, to ensure that the top of each of the 18 plants was at the same height.   The treatments were applied for seven weeks after which the plants began to flower and the experiment was ended.

2.1.4 Measurements: After the seven-week treatment period plants were destructively harvested. Shoot fresh mass (SFM), shoot dry mass (SDM), root dry mass (RDM), leaf area (LA), number of leaves, height to first bifurcation, number of nodes to first bifurcation, total height, stem diameter at the cotyledon and sixth nodes, relative chlorophyll levels (SPAD), and number of flower initials were all measured the day of harvest.

2.2 Fruit Production Study

2.2.1 Plant Material Preparation: Seeds of *Capsicum annum* 'California Wonder' (Lake Valley Seed Company Inc., Boulder, CO) were sown in mineral wool starter plugs (Grodan AO, Rockwool BV, Roermond, NL), placed in a germination tray, covered with a humidity dome, and placed in a controlled environment growth chamber (Environmental Growth Chambers, Chagrin Falls, OH. USA). The chamber was maintained at a constant 26°C during the germination period, with a 16-h photoperiod, relative humidity of 65%, and ambient CO_2 (chamber had CO_2 monitoring but no active control; levels ranged between 380-420 ppm). After two weeks, 10 seedlings were selected for uniformity and transplanted into 1.67L pots with media prepared as described in section 2.1.1. After 8 weeks' growth in the pots, all plants were pruned to open up the center of the plant to allow proper air movement.   The leaf area, fresh mass, dry mass, and flowers on the removed tissue were measured and included in the final tally for each plant.

2.2.2 Layout: The light distribution in the chamber used for this study varied significantly along the long axis of the chamber, ranging from 240 -350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) at canopy height; blocking on light intensity was implemented to accommodate the lack of uniformity. Pots were randomly assigned to one of five block positions within the growth chamber. Treatment levels (1-control; 2-mechanically stimulated) were randomly

144 assigned to one of the two positions within each block in a randomized complete block design
145 structure.

146

147 ***2.2.3 Treatment Application***: Mechanical stimulation (MS) was initiated after a 1-week
148 acclimation period following transplanting into 1.67 L pots. The MS was applied once daily in the
149 morning within 2-3 hours of the lights coming on for the duration of the experiment (11-weeks).
150 Once the plants had bifurcated, each of the most recently matured internodes on each branch
151 received MS, with the application per internode reduced to 10 strokes.   In order to avoid
152 confounding the MS dwarfing effect with the amount of incident light between the treatments,
153 the MS plants were placed on scissor lifts (Fisherbrand Lab Jacks, Fischer Scientific) to ensure that
154 the top of the MS plant was at the same canopy height as the control plant in each block. As the
155 plants began to branch, the uppermost internode on each primary branch received the MS. The
156 MS was only applied once daily during this trial, with treatments applied between 09:00-10:00
157 for the 11-week duration (12-weeks total in pots) of the trial.

158

159 **2.2.4 Measurements**:   After 12 weeks the plants were destructively harvested.   Similar to the
160 previous experiment SDM, LA, height to first bifurcation, number of nodes to first bifurcation,
161 total height, diameter at first true leaf node, and SPAD readings were recorded. Additional fruit
162 number, total fruit fresh mass, total fruit volume, and total fruit dry mass data was recorded for
163 each plant.   Resources were insufficient to allow leaf counts or root measurements during this
164 study. Plants from each block were placed on a black drop cloth and photographed from above.
165 A 100-cm ruler was included in the frame to allow post-harvest measurement of shoot diameters
166 (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA).

167

168 **3. RESULTS**

169 **3.1 VEGETATIVE STUDY**

170 Significant reductions were observed for all growth metric, although the shoot to root dry mass
171 ration did not differ (Fig. 1 A-H). The relative chlorophyll levels (SPAD) were significantly greater

172 in MS leaves (Fig. 2). The stem thickness of MS plants increased at the first node, relative to the

173 control plants, but the difference was reversed at the sixth node (Fig. 3)

174

175 **3.2 FRUITING STUDY**

176 The reductions in plant metrics observed in the vegetative study were not observed at the time

177 of harvest of mature, fruit bearing plants, with the notable exception of total plant height (Fig. 4).

178 The reduced number of flowers observed in the vegetative study was also noted in the fruiting

179 study with significantly fewer fruit being produced by the MS plants (Fig.5 A). Although there were

180 fewer flowers and resulting fruit produced, the total fruit volume, fresh mass and dry mass were

181 not significantly different between the control and MS groups (Fig. 5 B-D)

182

183 **4. CALCULATIONS**

184 Data from the fruiting trial were used as the basis for volume use efficiency (VUE) calculations.

185 The calculations focus on vertical components of VUE as crown diameter (area utilization),

186 reductions, although statistically significant, were not considered practically significant except on

187 extremely large scales; scales not likely to be realized in any practical spaceflight application.   It

188 is assumed that the vertical use improvements are additive with respect to VUE. It is accepted

189 that the following calculations are relatively simplistic in that they assume static interactions

190 between plants in terms of light competition and other environmental factors.   In the growth

191 study from which the data were gathered, care was taken to ensure a uniform access to light

192 although neighbour shading did occur. In less regulated systems some plants height

193 heterogeneity will increase resulting in further variation through shading and other competitive

194 effects.   Regardless, the exercise is valuable for highlighting the potential for using MS as a tool

195 for improving VUE in controlled environment agricultural systems.

196

197 **4.1 Vertical Use Calculations**

198 The mean shoot heights for the control and MS plants were 59.0 ± SD 1.4 cm and 47.2 ± SD 1.5

199 cm respectively (Figure 4C), which translates to a 20% plant height reduction. For the purposes of

200 this calculation the mean plus the standard deviation will be used in order to buffer the crop

201 variance. Assuming that in a stacked plant production system there is a total fixed height
202 requirement for lighting and rooting hardware of 30 cm, then the total vertical distance required
203 for control and MS plants is approximately 90.4 cm (60.4 + 30 cm) and 78.7 cm (48.7 + 30 cm)
204 respectively.   This represents an overall reduction in system height of 12.9% under MS. Carrying
205 this calculation forward, in the total height required to accommodate six stacked trays of control
206 plants (6 x 90.4 cm = 542.4 cm; round to 555 cm), one additional layer could conceivably be
207 included, if MS were employed (7 x 78.7 cm = 550.9 cm; Figure 6). Clearly this example is not
208 feasible in current spaceflight scenarios given the nearly 6 m vertical distance required to realize
209 the additional layer of plants; however, it is relevant to vertical farming in terrestrial settings
210 where significant production increases could be achieved. Recognizing this spaceflight limitation,
211 it still may be possible to grow plants otherwise unsuited for spaceflight production systems (e.g.,
212 Lada, Veggie, or proposed "salad machine" concepts) based on their crown architecture under
213 conditions where MS is absent[24]. Applying MS to these plants may prevent them from outgrowing
214 the plant production hardware, making them viable test species.
215
216 **5. Discussion**
217 Mechanical stimulation of *Capsicum annum* (cv California Wonder) resulted in significant
218 reductions in overall plant height in both the vegetative and fruiting study (Fig. 1H, 4C).   The
219 reductions were sufficient enough to realize improved VUE potential in life-support and other
220 vertically integrated production systems (Fig. 6), although the mode of that improvement differs
221 between terrestrial and spaceflight applications. The potential for improving VUE is greatest in
222 terrestrial settings where large volumes (e.g., warehouse scale production facilities) can be
223 exploited, such as the scales modelled in Fig. 6.   Long term space applications, such as a growth
224 chamber system on the Lunar or Martian surface, could also realize these VUE improvements. In
225 the near-term, spaceflight cropping system applications will be tightly constrained in the vertical
226 dimension, as well as the horizontal.   This said, MS in concert with other interventions such as
227 root restriction[18] or on its own, could be used to expand the species options for existing plant
228 production hardware (e.g., Veggie) by reducing the vertical space requirements for typically taller
229 crops such as *Capsicum spp.*

230

231   Crown diameter was also significantly reduced in the presented fruiting study, but unlike other

232   horticultural interventions (e.g., root restriction) examined by the authors[18], the reductions were

233   not sufficient in terms of area utilization to justify an increased planting density under conditions

234   of MS. The observed crown diameter reductions would only result in improved plant densities on

235   scales currently impractical for both terrestrial and spaceflight applications (e.g., 12 m wide

236   production benches; calculations not shown).

237

238   Other vegetative production metrics were reduced under the MS treatment during the vegetative

239   experiment (Figure 1-3); however, those vegetative differences did not persist or become evident

240   in the fruiting trial (Fig. 4), with the notable exceptions of total height and stem thickness (Fig. 4H

241   and E). Some of the discrepancy between the vegetative and fruiting studies may lie in an increase

242   in light competition/shading effects.   During the fruiting study, there was insufficient room at

243   maturity to exclude all incidence of shading between neighbouring plants.   This increased light

244   competition may have dampened some of the thigmomorphogenic effects through shade

245   adaptation responses which tend to elongate plants and increase leaf area[25,26].

246

247   In addition to being able to squeeze crop plants into a smaller volume and still maintain

248   productivity, MS could also be used as a countermeasure to ensure crop plants develop

249   structurally sound support tissue under microgravity conditions. Humans require significant

250   countermeasure interventions to reduce the negative impacts of microgravity of bone and muscle

251   tissue [27]; it stands to reason that crop plants making up part of a bioregenerative life-support

252   system may also benefit from microgravity countermeasures. In the absence of a significant

253   gravity vector plant cell walls and, by elaboration, supporting tissues (e.g., branches supporting

254   fruit) can be modified, although consensus on the degree and direction of the modifications is

255   elusive[14,28-32].   Very little (if any) research has examined the effects of direct mechanical stimuli

256   on crop plants in a microgravity setting. Having said this, it should be noted that the vibrational

257   environment of space research platforms, such as the International Space Station (ISS), do impose

258   a certain baseline level of mechanical stimulation to all plant experiments, but it is low—typically

259 < 0.001 g acceleration. Cleary the approach taken for this study required human intervention for
260 each plant, which would translate into "crew time" in space.   But automated systems for
261 applying thigmo- or seismic- stimuli might be envisioned.   For example, allowing canopies to
262 growth through a grid and mechanically shaking the grid each day to stimulate all the plants at
263 once But such approaches would require validation for efficacy.

264

265 Given the importance that crop plants will play in the future of human exploration, it is imperative
266 that attention be directed to all the various spaceflight environment parameters that will
267 influences the ability of the crops to deliver their life-support functions. Concurrently, potential
268 interventions, such as MS, that could contribute to improvements in VUE as well as providing
269 countermeasures to the rigours of the spaceflight environment should be considered.

270

271 **6. CONCLUSIONS**
272 Thigmomorphogenesis can be utilized to improve volume utilization efficiency in peppers
273 (*Capsicum annum* cv. California Wonder), a candidate crop for fresh food production in space.
274 The effect occurred primarily through a reduction in average plant height.   Reductions in
275 vegetative growth metrics during the juvenile growth phase (growth leading up to and including
276 early anthesis) were not observed during the mature or fruiting phase, with the notable exception
277 of reduced plant height. Early flower production and fruit set was reduced under MS; however,
278 the total edible biomass was not reduced, with MS plants producing fewer but larger fruits. The
279 overall reduction in plant height due to MS was sufficient to realize theoretical improvements in
280 VUE for large vertical farming systems.   The reduced heights observed could improve VUE in
281 single tier spaceflight hardware (e.g., Veggie; Massa 2016 (this issue)) in that crops that would
282 not normally fit in these spaceflight systems may be accommodated if MS can be applied.
283 Although the potential for using MS to induce thigmomorphogenic phenotypes has long been
284 appreciated, it is only recently that the growth systems themselves could take advantage of the
285 modified crop architecture associated with MS. It is with this in mind that renewed attention
286 should be given to developing procedures for environmentally modifying crops for spaceflight
287 applications.

288

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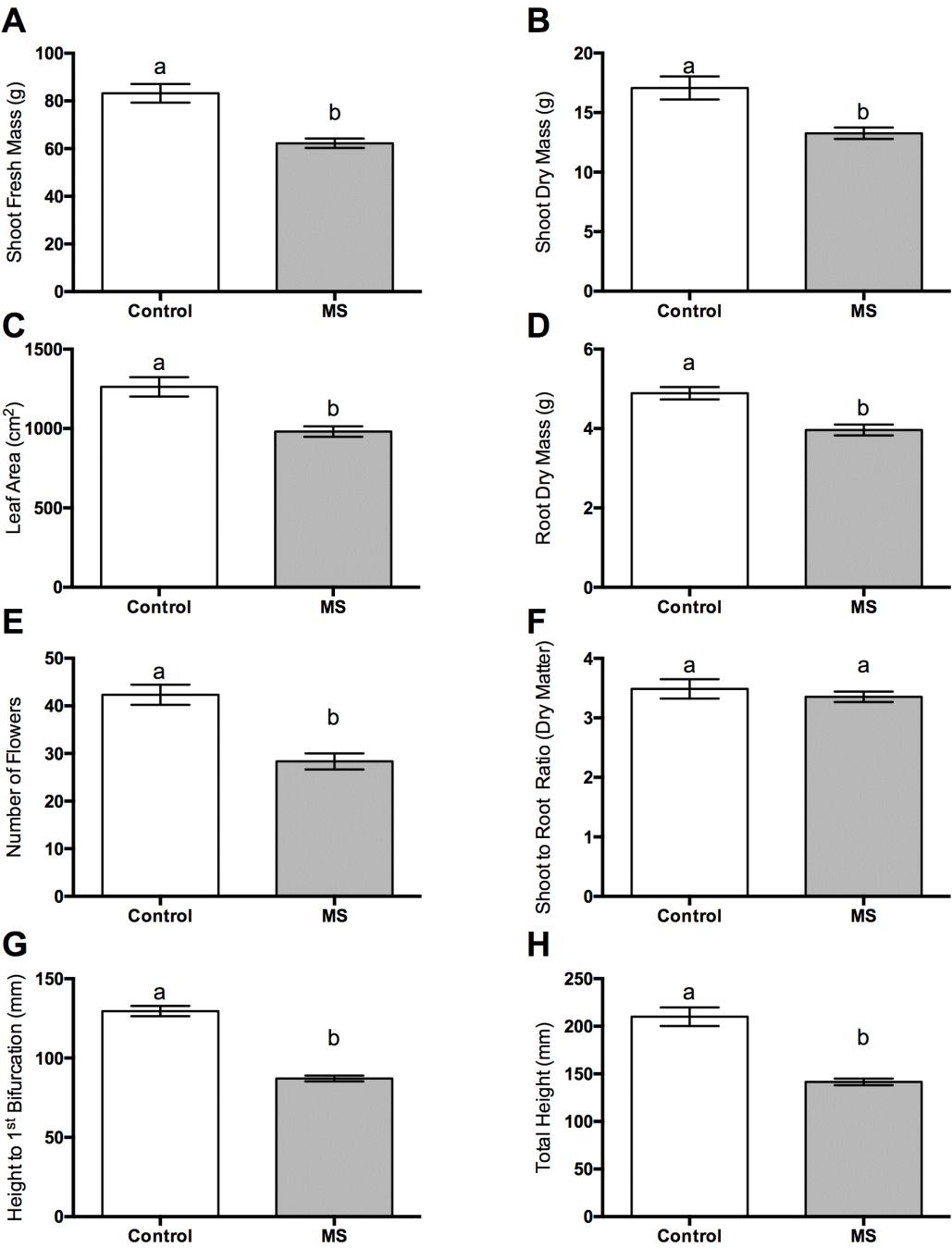
361   31(1):51-61.

362

363 *Figure 1: Plant growth response to mechanical stimulation during juvenile and early anthesis growth stages: (A) Shoot fresh mass;*
364 *(B) Shoot dry mass; (C) Leaf area; (D) Root dry mass; (E) Flower production; (F) Shoot to root dry matter ratio; (G) Height at the*
365 *first stem bifurcation; (H) Total height. Columns with the same letter appearing above do not differ at p≤0.05. Error bars are the*
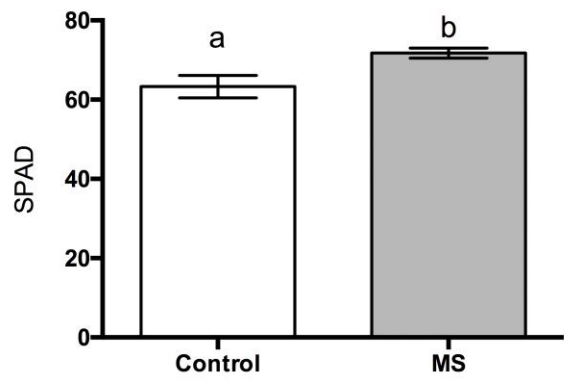366 *SEM.*

367

368 *Figure 2: Relative chlorophyll levels (SPAD) in the last fully expanded leaf under control and mechanical stimulation treatments.*
369 *Columns with the same letter appearing above do not differ at p≤0.05. Error bars are the SEM.*
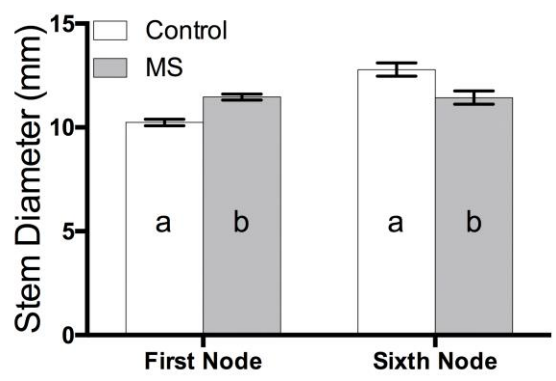
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371 *Figure 3: Stem diameter at the first and sixth node for control and mechanically stimulated pepper plants. Bars within each grouping*
372 *(e.g., first node) with the same letter do not differ at p≤0.05. Error bars are SEM.*
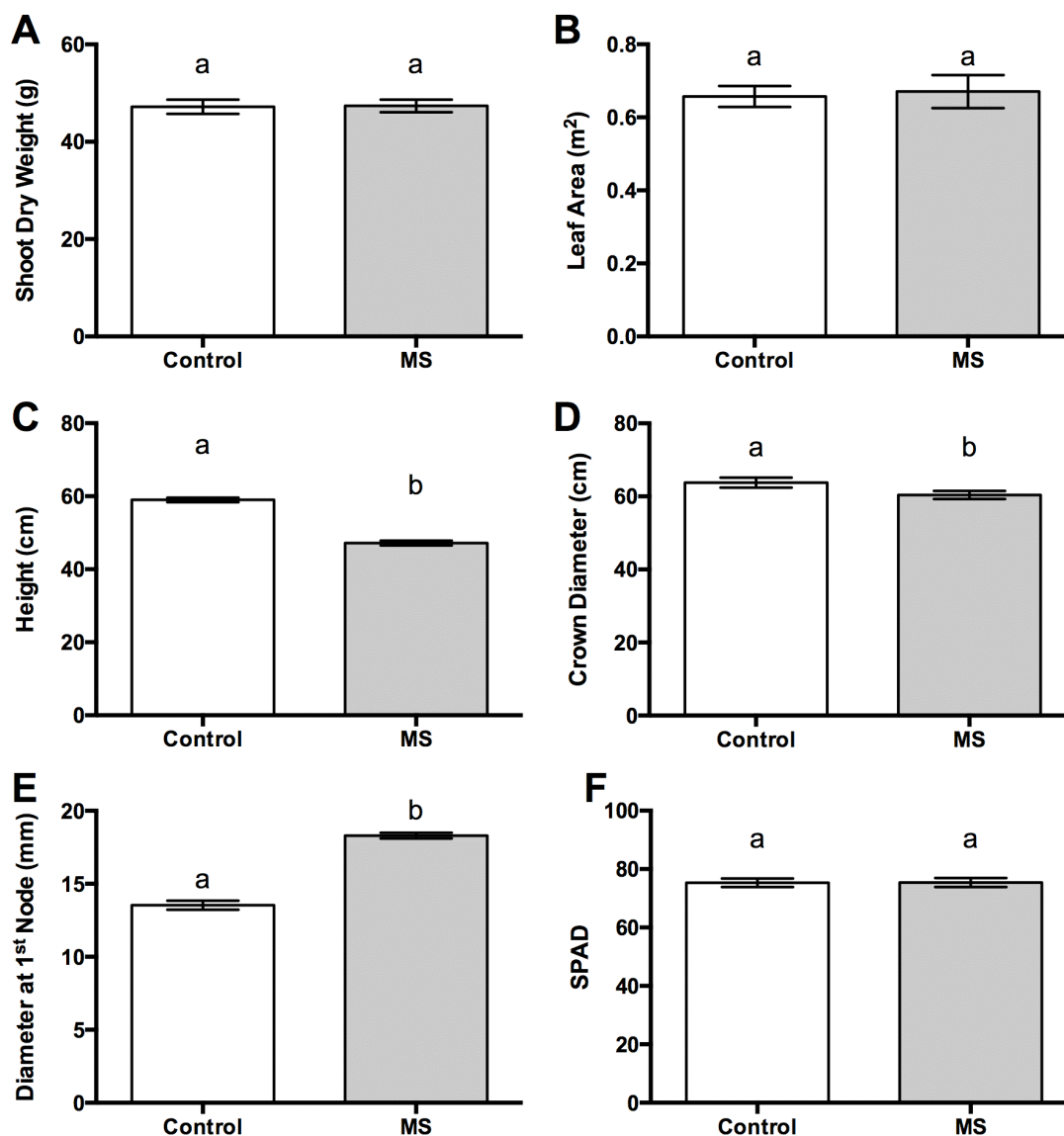
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374 *Figure 4: Vegetative shoot metrics for control and mechanically stimulated pepper plants during fruit set and maturation: (A) Shoot*

375 *dry mass; (B) Leaf area; (C) Total height; (D) Crown diameter; (E) Stem diameter at the first node; (F) Relative chlorophyll level*

376 *(SPAD). Columns with the same letter appearing above do not differ at p≤0.05. Error bars are the SEM.*
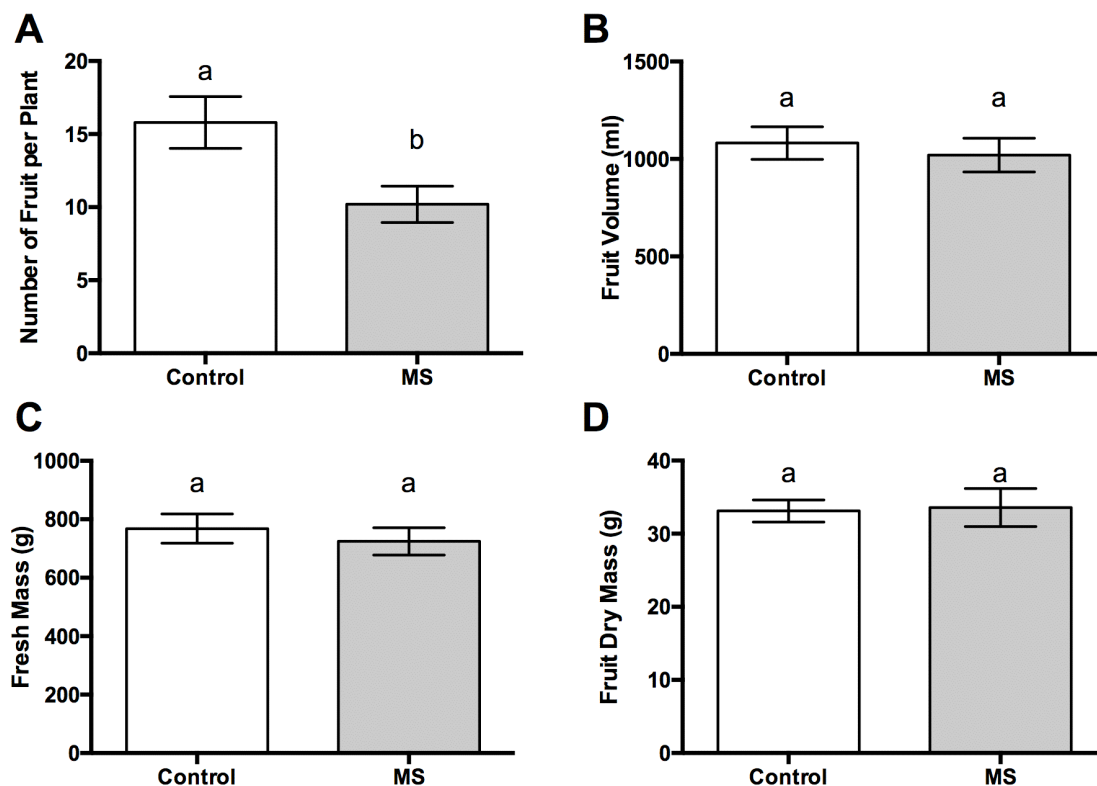
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378

Figure 5: Fruit production metrics for control and mechanically stimulated pepper plants: (A) Average number of mature fruit per
plant; (B) Mean total fruit volume per plant; (C) Mean total fruit mass per plant; (D) Mean total fruit dry mass per plant. Columns
with the same letter appearing above do not differ at p≤0.05. Error bars are the SEM.
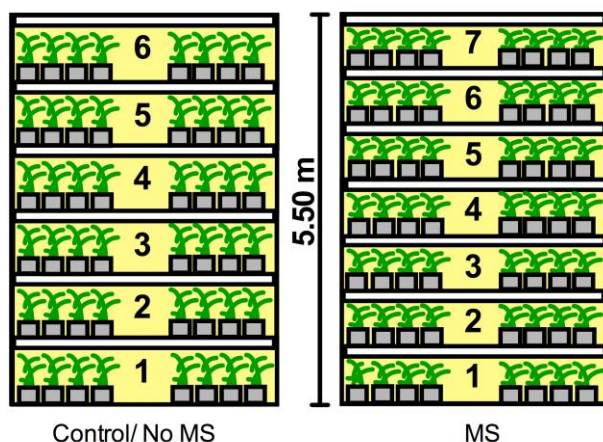
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Figure 6: Theoretical Volume Utilization Efficiency improvement potential in stacked crop production system based on the mean
height reductions observed in the fruiting experiment presented.